Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial[1–4]

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ABSTRACT

Background: Studies suggest cardioprotective benefits of dark chocolate containing cocoa.

Objective: This study examines the acute effects of solid dark chocolate and liquid cocoa intake on endothelial function and blood pressure in overweight adults.

Design: Randomized, placebo-controlled, single-blind crossover trial of 45 healthy adults [mean age: 53 y; mean body mass index (in kg/m^2): 30]. In phase 1, subjects were randomly assigned to consume a solid dark chocolate bar (containing 22 g cocoa powder) or a cocoa-free placebo bar (containing 0 g cocoa powder). In phase 2, subjects were randomly assigned to consume sugar-free cocoa (containing 22 g cocoa powder), sugared cocoa (containing 22 g cocoa powder), or a placebo (containing 0 g cocoa powder).

Results: Solid dark chocolate and liquid cocoa ingestion improved endothelial function (measured as flow-mediated dilatation) compared with placebo (dark chocolate: 4.3 ± 3.4% compared with −1.8 ± 3.3%; $P < 0.001$; sugar-free and sugared cocoa: 5.7 ± 2.6% and 2.0 ± 1.8% compared with −1.5 ± 2.8%; $P < 0.001$). Blood pressure decreased after the ingestion of dark chocolate and sugar-free cocoa compared with placebo (dark chocolate: systolic, −3.2 ± 5.8 mm Hg compared with 2.7 ± 6.6 mm Hg; $P < 0.001$; and diastolic, −1.4 ± 3.9 mm Hg compared with 2.7 ± 6.4 mm Hg; $P = 0.01$; sugar-free cocoa: systolic, −2.1 ± 7.0 mm Hg compared with 3.2 ± 5.6 mm Hg; $P < 0.001$; and diastolic: −1.2 ± 8.7 mm Hg compared with 2.8 ± 5.6 mm Hg; $P = 0.014$). Endothelial function improved significantly more with sugar-free than with regular cocoa (5.7 ± 2.6% compared with 2.0 ± 1.8%; $P < 0.001$).

Conclusions: The acute ingestion of both solid dark chocolate and liquid cocoa improved endothelial function and lowered blood pressure in overweight adults. Sugar content may attenuate these effects, and sugar-free preparations may augment them. *Am J Clin Nutr* 2008;88:58–63.

INTRODUCTION

Recent studies suggest vascular health benefits associated with dark chocolate (1–3). This is of particular interest given the popularity of chocolate and the potential it offers for people to derive health benefits from a food category associated with high palatability and indulgence. Evidence is accumulating that some forms of cocoa and chocolate, rich in flavonoids, may have the potential to improve cardiovascular health (4). The beneficial cardiovascular effects of flavonoids are attributed to their ability to improve endothelial function, by activation of the nitric oxide (NO) synthase system, their natural antioxidant properties, and their ability to decrease blood clotting by inhibiting platelet activation and aggregation (1, 4–9).

Endothelial function refers to arterial vasomotor responses mediated by the release of chemicals, including NO (vasodilating) and endothelin (vasoconstricting), from the vascular endothelium (10). Impaired release of NO results in endothelial dysfunction, in which vessels tend to constrict and impede flow in response to stimuli that should lead to dilatation and flow augmentation (11). Endothelial function can be assessed noninvasively through the induction of hyperemic flow and sheer stress to stimulate NO release (12). Because of the strong correspondence between peripheral and coronary endothelial responses (13, 14), measurement of endothelial-dependent flow-mediated dilation of the brachial artery with the use of high-resolution ultrasound scanning has become a standard research assessment method (15).

Although dark chocolate appears to improve endothelial function, several studies report impairment of endothelial function with acute glucose loading (16). To our knowledge, no study has directly compared the vascular effects of regular compared with sugar-free cocoa ingestion or directly compared the vascular effects of solid dark chocolate with liquid cocoa. We therefore performed a randomized, placebo-controlled, single-blind crossover trial to examine the acute effects of cocoa consumption in solid and liquid (regular and sugar-free) preparations on endothelial function and blood pressure in apparently healthy persons at risk of cardiovascular disease because of elevated body mass index (BMI; in kg/m^2).

SUBJECTS AND METHODS

Subjects

A total of 45 healthy adults (10 men and 35 women) were recruited from the Lower Naugatuck Valley, CT, through newspaper advertisements and posters at frequented sites. Those responding ($n = 129$) were prescreened with the use of a semistructured telephone interview. Eligible participants

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were nonsmoking adults aged 30–75 y with a BMI between 25 and 35 and a waist circumference > 88 cm in women and 102 cm in men. Exclusion criteria included a current eating disorder, diagnosed coronary artery disease (CAD), diabetes mellitus, sleep apnea, pregnancy, use of insulin- or glucose-sensitizing medication, restricted diets by choice (ie, vegan, carbohydrate-restricted, etc), or an allergy to cocoa or chocolate.

Those meeting initial prescreening criteria ($n = 75$) underwent a clinical screening examination consisting of height, weight, BMI, and blood pressure measurements and laboratory testing consisting of total cholesterol, HDL, LDL, triacylglycerols, and fasting blood glucose. The study protocol and consent form were approved by the Griffin Hospital (Derby, CT) Institutional Review Board and the Yale University (New Haven, CT) Human Investigation Committee. Written informed consent was obtained, and all subjects received monetary compensation for their participation. Subject participation and flow are shown in **Figure 1**.

Study design, randomization, and sample size

This study was a randomized and placebo-controlled crossover trial, with investigators involved in data collection and analysis blinded to treatment assignment. In phase 1, 45 subjects were randomly assigned to receive 1 of the 2 possible sequences of 74 g solid dark chocolate (containing 22 g cocoa powder) or 74 g placebo (containing 0 g cocoa powder) (**Table 1**). In phase 2, subjects were randomly assigned to 1 of the 6 possible sequence permutations of sugar-free cocoa (2 cups containing 22 g cocoa powder), sugared cocoa (2 cups containing 22 g cocoa powder), or a placebo (2 cups providing 0 g cocoa powder) (Table 1); each cup was prepared in 8 oz (240 mL) of hot water. The cocoa with sugar contained 45.3 g sugar/serving, whereas the sugar-free version was sweetened with small amounts of vanillin, acesulfame-potassium, and aspartame.

Subjects underwent endothelial function testing after an 8-h, overnight fast and then 2 h after each treatment. Serum concentration of the flavonoid epicatechin was shown to be highest 2 h

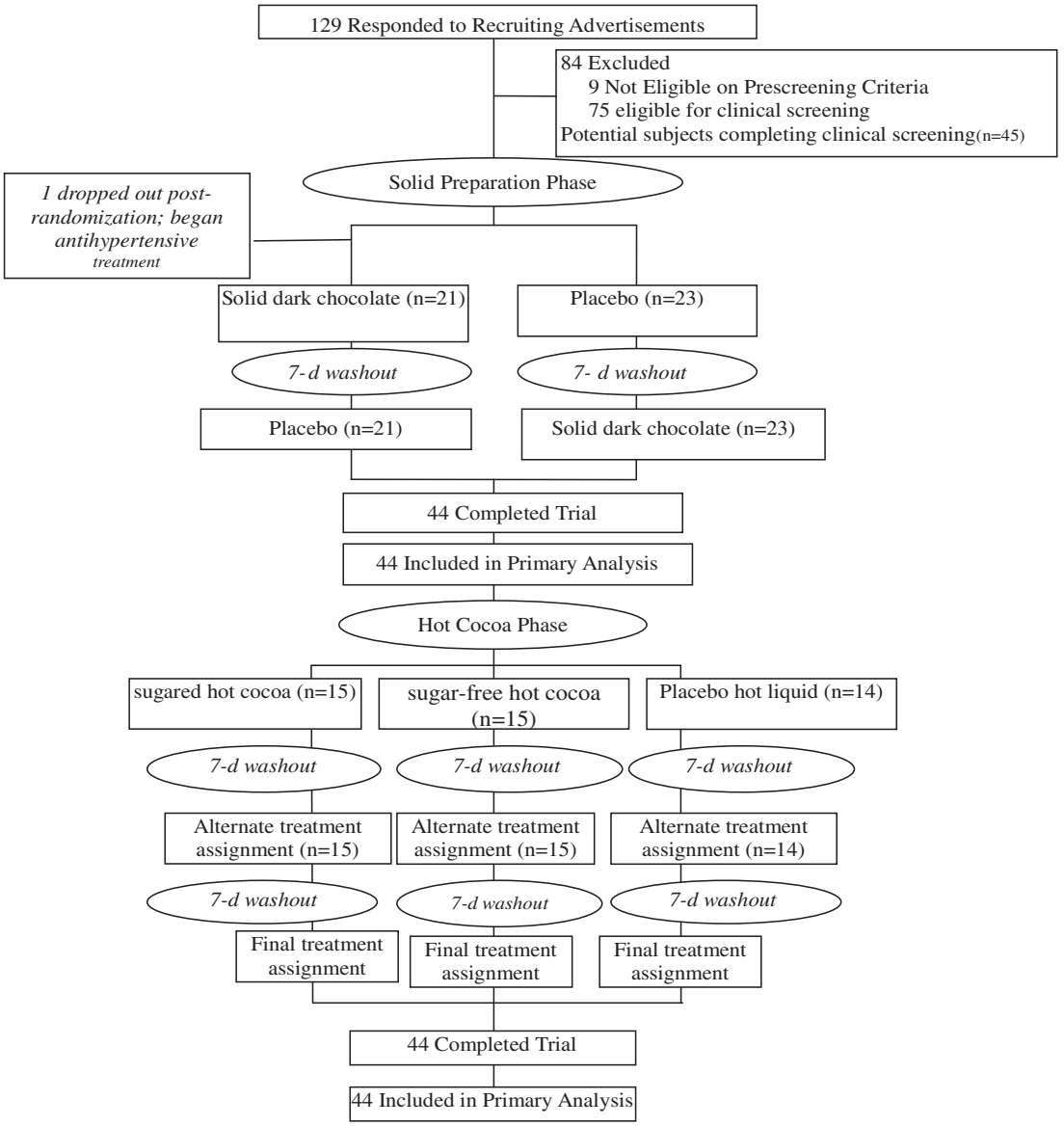

FIGURE 1. Flow of participants through the trial.

TABLE 1
Composition of test products[1]

Content	Placebo chocolate	Solid dark chocolate	Sugar-free cocoa	Sugared cocoa	Placebo cocoa
Weight (g)[2]	74	74	23.4	114.9	125.3
Cocoa powder (g)	0	22	22	22	0
Energy (kcal)	389	327	90	460	500
Total fat (g)	22	27	2	2	2
Carbohydrates (g)	44	39	12	104	110
Protein (g)	6	6	6	6	8
Sodium (mg)	97	4	110	110	410
Potassium (mg)	306	366	334	334	512
Calcium (mg)	215	33	33.4	33.4	314
Magnesium (mg)	19	119	133.6	133.6	38
Catechin (mg)	0	10.4	20.9	20.9	0
Epicatechin (mg)	0	21.5	48.4	48.4	0
Procyanidin dimer (mg)	0	81.4	92.0	92.0	0
Procyanidin trimer (mg)	0	67.3	98.1	98.1	3.3
Procyanidin tetramer (mg)	0	37.0	30.6	30.6	0
Procyanidin pentamer and hexamer (mg)	0	67.0	54.8	54.8	5.5
Total procyanidins (total flavanols) (mg)	0	821	805.2	805.2	8.8
Theobromine (mg)	1.5	525	436	436	0
Caffeine (mg)	3.7	44	28.1	28.1	0

[1] Energy and nutrient data of the tested products are provided by the Hershey Company.
[2] Refers to total product weight.

after cocoa consumption (6, 17). Each treatment was followed by a 7-d washout period before the subsequent treatment was administered.

Weight, height, and blood pressure were measured at each visit. Participants' baseline data were collected for all outcome measurements before the first treatment and at the end of the washout period between treatment assignments. Subjects were instructed to maintain their usual diet except to refrain from flavonoid-rich food and beverages, alcoholic beverages, vitamin supplements, and certain medications for a 7-d run-in period before starting the study. Participants were also instructed to fast overnight before each endothelial function scan. They were advised to maintain their usual physical activity during the study period.

The sample size was determined to allow for ≈20% attrition and noncompliance and to provide ≥80% power to detect a minimal difference of 3.0% in flow-mediated dilatation (FMD) between cocoa and placebo with maximum allowable type I error of 5% adjusted for 3 pairwise comparisons. An SD of 6.3% for FMD was used to compute the sample size.

Vascular reactivity testing: brachial artery reactivity studies

Each vascular reactivity test consisted of pre- and postprandial brachial artery reactivity studies. Endothelial function was measured noninvasively in the right brachial artery by a high-frequency ultrasound scanning machine (Sonos 4500; Phillips Medical Systems, Andover, MA) in accordance with published guidelines (15). Subjects were required to lie at rest in the quiet, temperature-controlled, softly lit room for ≥15 min before scanning was initiated. The baseline diameter of the brachial artery was measured from 2-dimensional ultrasound scanning images with the use of a high-frequency, 10–15 MHz, vascular ultrasound scanning transducer (15-6L L7540 linear array transducer; Phillips Medical Systems). Arterial flow velocity was measured by a pulsed Doppler scan signal at a 70-degree angle to the vessel, with the range gate in the center of the artery. Flow is determined by multiplying the arterial cross-sectional area (πr^2) by the Doppler scan flow velocity. The timing of each image frame with respect to the cardiac cycle is determined with simultaneous electrocardiographic gating during image acquisition by the high-quality mainframe ultrasound scanning system. Measurements were taken from the anterior to the posterior M line in diastole. The brachial artery was imaged at a location 3–7 cm above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous 2-dimensional gray-scale imaging. The transmit (focus) zone was set to the depth of the near wall because of difficulty in differentiating the near from the far wall M line (the interface between media and adventitia). Images were acquired on videotape and magnetic optical disk for evaluation and analysis. Diameter was obtained from M line to M line, over a consistent segment of vessel at least 10–15 mm in length. To create a flow stimulus in the brachial artery, a sphygmomanometer (blood pressure cuff) was placed on the upper arm proximal to the transducer. The cuff was inflated for 5 min. Repeat scans were obtained at 15, 60, and 120 s after deflation. At each scanning interval, both cross-sectional vessel diameter and flow velocity were recorded. Measures of vessel diameter and flow velocity were obtained by a single dedicated vascular clinical research specialist blinded to subject treatment status. Velocity measures were generated automatically, whereas the arterial diameter was measured at a fixed distance from an anatomical marker, such as a bifurcation, with ultrasonic calipers recorded on magnetic-optical disk. A random sample of 30 brachial artery reactivity studies was provided to the clinical research specialist for a blinded second reading. The resultant coefficient of intraobserver reliability was 0.95.

Outcome measures

Endothelial function

Endothelial function was measured as FMD, the percentage of change in brachial artery diameter from before cuff inflation to 60 s after cuff release. In addition to brachial diameter at 60 s after cuff release, flow after cuff deflation within the first 15 s was used as an indicator of stimulus strength, hyperemic flow being the stimulus for endothelial reactivity. To account for potential variability in stimulus strength, FMD was divided by flow at 15 s after cuff deflation to create a stimulus-adjusted response measure. The primary study outcome measure was the difference in FMD change before and after each treatment.

Blood pressure

Blood pressure was determined with the use of the Datascope Accutorr Plus automatic digital blood pressure device (Datascope Corp, Mahwah, NJ) with the subject supine after a 5-min period of rest. Both systolic and diastolic pressures were calculated as the mean value of 2 readings 5 min apart.

Statistical analysis

Repeated-measures analysis of variance (ANOVA) was used to assess differences in intraindividual responses across treatments. Paired *t* tests were also used to compare baseline mean values of all outcome measures (ie, endothelial function and blood pressure) among participants by intervention assignment (ie, cocoa or placebo). Tukey's test was used for post hoc analysis of the repeated-measures ANOVA to correct for experimentwise error rate. The combined effect of independent variables (age, race, BMI, hypertensive, and dyslipidemia) and treatment assignment on all outcome measures was assessed with multivariable models with the use of ANOVA. All analyses of endpoints were based on the intention-to-treat principle. Data were analyzed with the use of SAS software for WINDOWS version 9.1 (18). Results are expressed as means ± SD in text and tables.

RESULTS

Study participants

Of the 75 subjects screened, the first 45 to qualify participated in the study. All but 1 of the 45 randomly assigned subjects completed the study; 1 subject was removed after being prescribed antihypertensive medications by her primary care provider (Figure 1). The mean age of participants was 53 y and the average BMI was 30; 78% of subjects were women. Endothelial function and blood pressure were comparable ($P > 0.05$) before each treatment assignment. Baseline characteristics are shown in **Table 2**.

Phase 1: solid dark chocolate

After consumption of a single dose of solid dark chocolate, FMD improved from baseline compared with placebo (4.3 ± 3.4% compared with −1.8 ± 3.3%; $P < 0.001$). Single-dose consumption of solid dark chocolate also lowered blood pressure from baseline compared with placebo (systolic: −3.2 ± 5.8 mm Hg compared with 2.7 ± 6.6 mm Hg; $P < 0.001$; diastolic: −1.4 ± 3.9 mm Hg compared with 2.7 ± 6.4 mm Hg; $P < 0.001$) (**Table 3**). Findings persisted after controlling for age, race, BMI, hypertensive, and dyslipidemia of participants.

TABLE 2
Demographic characteristics and baseline values

Variable	Value
Age (y)	52.8 ± 11.0[1]
Dislipidemia	3 (6.7)[2]
Hypertensive	1 (2.2)
Medication use[3]	4 (8.9)
BMI (kg/m^2)	30.1 ± 3.3
Weight (kg)	81.3 ± 10.2
Room temperature (°F)	72.0 ± 0.2

[1] $\bar{x}$ ± SD (all such values); $n = 45$.
[2] n; percentage in parentheses (all such values).
[3] Included Lipitor, Avapro, Lotensin, and verapamil.

Phase 2: liquid cocoa

Consumption of a single dose of sugar-free and sugared cocoa improved FMD from baseline compared with placebo (5.7 ± 2.6% and 2.0 ± 1.8% compared with −1.5 ± 2.8%; $P < 0.001$). The magnitude of improvement in FMD after consumption of sugar-free cocoa was significantly greater than that after sugared cocoa consumption (5.7 ± 2.6% compared with 2.0 ± 1.8%; $P < 0.001$) (**Table 4**). In addition, single-dose consumption of sugar-free cocoa led to a significant reduction in both systolic and diastolic blood pressures from baseline compared with placebo (systolic: −2.1 ± 7.0 mm Hg compared with 3.2 ± 5.6 mm Hg; $P < 0.001$; diastolic: −1.2 ± 8.7 mm Hg compared with 2.8 ± 5.6; $P = 0.014$). No change was observed in blood pressure after the consumption of sugared cocoa compared with placebo.

DISCUSSION

The acute ingestion of either solid dark chocolate or liquid cocoa significantly improves endothelial function and lowers blood pressure in healthy, overweight adults. These effects are significantly greater for sugar-free than for regular cocoa. To our knowledge, this is the first study to examine the acute effects of sugar-free cocoa on endothelial function and blood pressure and to examine the effects of solid chocolate and liquid cocoa in the same subjects.

The improvement in endothelial function observed after cocoa consumption in liquid and solid forms is most likely related to elevated concentrations of plasma epicatechin, which increases endothelium-derived vasodilators that result in improved endothelial function. In addition, the procyanidins in cocoa were shown to increase NO production by activating endothelial NO synthase and inducing endothelium-dependent relaxation (8).

Our results of cocoa consumption in liquid and solid preparations on endothelial function are consistent with most studies done on dark chocolate consumption in several different population groups. Engler et al (6) and Fisher et al (5) showed that flavonoid-rich dark chocolate improves endothelial function in healthy adults. Grassi et al (19) reported improved endothelial function in persons with hypertension after dark chocolate ingestion over the course of 2 wk. Heiss et al (20) found that acute consumption of cocoa in smokers led to an increase in reactive NO species and improvement in endothelial function, whereas others found improvements in brachial artery flow in hypercholesterolemic, postmenopausal women (21). However, Farouque et al (22) did not observe any changes in endothelial function with either acute or sustained consumption of a cocoa beverage in a

TABLE 3
Chocolate: change from baseline in endothelial function and blood pressure[1]

Variable	Solid dark chocolate	Placebo
Endothelial function		
Flow-mediated dilation (%), baseline	7.4 ± 3.6	9.1 ± 4.8
ΔFlow-mediated dilation (%)	4.3 ± 3.4[2]	−1.8 ± 3.3
Stimulus-adjusted response measure, baseline	0.08 ± 0.08	0.07 ± 0.17
ΔStimulus-adjusted response measure	0.04 ± 0.10	0.01 ± 0.18
Blood pressure		
Systolic blood pressure (mm Hg), baseline	124.8 ± 17.0	122.8 ± 15.2
ΔSystolic blood pressure (mm Hg)	−3.2 ± 5.8[2]	2.7 ± 6.6
Diastolic blood pressure (mm Hg), baseline	68.6 ± 11.8	69.9 ± 11.7
ΔDiastolic blood pressure (mm Hg)	−1.4 ± 3.9[2]	2.7 ± 6.4

[1] All values are $\bar{x}$ ± SD; $n = 44$. Δ, Change from baseline.
[2] Significantly different from placebo, $P < 0.001$ (repeated-measures ANOVA).

population with known CAD. This anomalous result could be explained by the fact that once clinical CAD is diagnosed, the changes in the endothelium are too advanced to be ameliorated by cocoa consumption.

Previous studies examining the effect of cocoa ingestion on blood pressure report mixed results. Grassi et al (19) found a significant decrease in both systolic and diastolic blood pressures after dark chocolate consumption in 10 subjects with grade 1 hypertension while also reporting a similar decrease in blood pressure in 15 healthy persons after 7 consecutive days of cocoa consumption. Taubert et al (23) reported a significant decrease in both systolic and diastolic blood pressure after ingestion of flavonol-rich cocoa for 10 d in 13 otherwise healthy persons with isolated systolic hypertension. Recent studies (24, 25) have shown habitual amounts of cocoa lowered blood pressure in a sample of healthy persons with above-optimal blood pressure. In a cross-sectional study, long-term cocoa consumption was found to be inversely associated with blood pressure and 15-y cardiovascular and all-cause mortality (26). However, in clinical trials, Engler et al (6) and Fisher et al (5) were unable to document any blood pressure change in young, healthy, normotensive persons after dark chocolate consumption over the course of 2 wk. Taken together, this may indicate that cocoa and dark chocolate may aid in normalizing elevated blood pressure while helping to maintain healthy blood pressure in those with blood pressure in the normal range.

Previous studies have indicated that postprandial hyperglycemia and acute glycemic load result in inflammatory responses and endothelial dysfunction (27). Diets with a high glycemic index were shown to increase the risk of CAD and to affect endothelial function adversely by several mechanisms, including oxidative stress, inflammatory factors, protein glycation, LDL oxidation, and procoagulatory and antifibrinolytic activities (28). Lower glucose and insulin concentrations are associated with an improved risk profile for CAD, including improved HDL cholesterol, oxidative status, and endothelial function (29, 30).

Several researchers have examined the effect of an oral glucose load, which mimics a postprandial state, on circulating insulin and glucose concentrations and vascular arterial function in healthy persons (normal blood glucose concentrations). Akbari et al (16) and Title et al (31) report impaired endothelial function after an oral glucose overload in healthy subjects. The researchers hypothesize that hyperglycemia suppresses endothelium-dependent vasodilation, possibly by production of oxygen-free radicals, which decreases the bioavailability of NO. In contrast, Siafarikas et al (32) report no change in vascular function in 39, nonobese, healthy subjects after oral glucose tolerance testing.

Our study showed benefits in endothelial function after the acute consumption of cocoa; the improvements observed with the consumption of sugar-free cocoa were significantly greater than those seen with sugared cocoa. Both sugar and sugar-free cocoa had 22.0 g cocoa powder, containing 3282 mg polyphenols. Because the antioxidant composition of the 2 cocoas was the same, the greater effects of sugar-free cocoa can be attributed to the removal of the sugar. Thus, we can conclude that the polyphenols in chocolate, particularly flavanoids, improve endothelial function, whereas the sugar content attenuates this effect when consumed in liquid form.

The study has several limitations. The findings pertain to the acute ingestion of cocoa only. Study results could be influenced by potential confounders such as the association between nutrient intake and endothelial and physiologic responses, including

TABLE 4
Cocoa: change from baseline in endothelial function and blood pressure[1]

Variable	Sugar-free cocoa	Sugared cocoa	Placebo
Endothelial function			
Flow-mediated dilation (%), baseline	5.6 ± 2.6	5.8 ± 2.2	6.3 ± 2.9
ΔFlow-mediated dilation (%)	5.7 ± 2.6[2,3]	2.0 ± 1.8[2]	−1.5 ± 2.8
Stimulus-adjusted response measure, baseline	0.06 ± 0.05	0.06 ± 0.03	0.06 ± 0.08
ΔStimulus-adjusted response measure	0.04 ± 0.05[2]	0.02 ± 0.05[4]	−0.02 ± 0.08
Blood pressure			
Systolic blood pressure (mm Hg), baseline	123.4 ± 15.2	121.4 ± 15.5	121.3 ± 13.9
ΔSystolic blood pressure (mm Hg)	−2.1 ± 7.0[2]	0.9 ± 8.7	3.2 ± 5.6
Diastolic blood pressure (mm Hg), baseline	68.2 ± 13.8	67.7 ± 12.6	68.9 ± 12.7
ΔDiastolic blood pressure (mm Hg)	−1.2 ± 8.7[5]	1.7 ± 6.2	2.8 ± 5.6

[1] All values are $\bar{x}$ ± SD; $n = 44$. Δ, Change from baseline.
[2,4,5] Significantly different from placebo (repeated-measures ANOVA and Tukey's test): [2]$P < 0.001$, [4]$P < 0.01$, [5]$P < 0.02$.
[3] Significantly different from sugared cocoa, $P < 0.001$ (repeated-measures ANOVA and Tukey's test).

unmeasured or inaccurately measured dietary intake data, changes in physical activity, noncompliance, vasoactive medication use, and genetic factors. The measurements of endothelial function were made at a single time point, so they could have missed both temporal and maximal differences. The lack of the measurement of plasma catechin concentrations precludes knowing whether the effects were attributable to these components. The lack of dietary control and information about diet reduce the certainty that the effects seen were due exclusively to the interventions. However, subjects fasted overnight before receiving treatment assignment the following morning. Given this, and the fact that each subject served as his or her own control, effects of diet are likely to be minimal and nondifferential. Other limitations include limited generalizability because of the homogeneous population; the majority of enrolled subjects were white women working and living in close geographic proximity in the Naugatuck Valley, CT.

In conclusion, this study shows beneficial effects of acute consumption of cocoa in solid and liquid preparation on cardiac risk in overweight persons. This trial further suggests that the cardioprotective influence of acute cocoa ingestion is attenuated by the sugar content of cocoa-containing beverages and accentuated by the removal of sugar. Further investigation is clearly warranted to determine longer term effects of habitual solid and liquid cocoa ingestion, optimal dosing of chocolate for cardiovascular benefit, variation in beneficial effects among diverse populations, and, ultimately, the influence of dietary cocoa intake on cardiac events.

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The authors responsibilities were as follows—DLK (principal investigator): responsible for oversight of all study related activities, data analysis, and manuscript preparation: ZF: responsible for study management, ultrasound reading, data collection, and manuscript preparation; VYN: responsible for data analysis, interpretation, manuscript preparation, and critical review of the paper; AA: contributed to manuscript preparation; SD: contributed to protocol development and manuscript preparation. None of the authors had a personal or financial conflict of interest.

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